

ABSTRACT

A method is described for cleaving a nucleic acid substrate with a nucleic acid enzyme at a cleavage site comprising mixing the substrate with the enzyme, wherein the substrate includes a 7 nucleotide sequence with at least 6 nucleotides 3' to the cleavage site and at least 1 nucleotide 5' to the cleavage site and of formula:



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wherein each N is a nucleotide which may be the same or different, H is a nucleotide selected from the group consisting of A, U, C, and T, and is the site of cleavage, and H' is a ribonucleotide selected from the group consisting of A, U, and C, wherein (i) the first nucleotide 3' to the cleavage site is capable of forming a wobble pair with the enzyme, (ii) the second, third, fifth, and sixth nucleotides 3' to the cleavage site are capable of forming conventional Watson-Crick base pairs with the enzyme, (iii) the fourth nucleotide 3' to the cleavage site is capable of forming a non-conventional Watson-Crick base pair with the enzyme, and (iv) the first nucleotide 5' to the cleavage site does not form a base pair with the enzyme; and the enzyme comprises a substrate binding portion which is capable of base pairing to the 6 nucleotides 3' to the cleavage site of the substrate and which binding portion comprises the sequence:



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wherein each N is a nucleotide which may be the same or different, and X is a nucleotide selected from the group consisting of T, U, A, and G, whereby binding of the substrate to the enzyme effects cleavage of the substrate at the cleavage site.

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